HOST SPECIFICITY IN THE *RHIZOBIUM* SYMBIOSES OF TWO *ASPALATHUS* SPECIES AND AN INVASIVE ALIEN LEGUME, *ACACIA SALIGNA*.

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ABSTRACT

The symbiotic specificity of Aspalathus capensis and A.carnosa in relation to their geographical distribution, was investigated by growing plants in soil and in tubes containing soil solutions plus nitrogen-free nutrient solutions. Soils were collected from three sites within their distribution and two from 90km outside. Both species formed nodules in all three soils from within their distribution and A.capensis in one from outside. A.carnosa did not survive in tube culture. The possible role of host-specificity in the distribution of A.capensis and A.carnosa is discussed.

A widespread invasive alien legume, Acacia saligna . was grown in the same soils and soil solutions. It formed nodules only in the three soils from within the distribution of Aspalathus capensis. The degree of nodulation varied between these soils. The possible importance of host-strain specificity in the invasive ability of alien legumes is discussed.

Bacteria of the family Rhizobiaceae form a symbiosis with legumes. This involves an interaction with plant roots, resulting in the formation of root nodules, in which nitrogen fixation occurs. This interaction takes place in a species-specific way, which is genetically determined for both the host and the bacteria (Spaink et al 1987). Thus, a given host species can only be nodulated by a certain group of strains or genetic lines of bacteria. This group may be very diverse or highly restricted (Trinick et al 1968, Dreyfuss and Dommergues 1981, Glatzle et al 1986). Two short studies of Aspalathus linearis ssp. linearis showed symbiotic specificity at the generic level (Staphorst and Strijdom 1975) and at the species level (Deschodt and Strijdom 1976). However, these studies did not consider the natural distributions of the species investigated.

Devine (1984) suggested that the origin of natural species-strain specificity is the result of coevolution between local host ecotypes and indigenous Rhizobium strains. This results in interrelated distributions of host plants and their specific strains (Lie et al 1987). Ecological factors such as soil moisture, pH and temperature, influence the survival of rhizobia in soil (Woomer et al 1988) and differential tolerance of strains to these factors has been suggested to influence their distribution (Eaglesham et al 1987, Yousef et al 1987). Thus interrelated plant and Rhizobium strain distributions may be influenced by tolerance of the bacteria to soil factors, such as

pH. This is illustrated in the case of Hedysarum coronarium where both the plant and its highly specific Rhizobium strain (Glatzle et al 1986) are restricted to calcareous soils in southern Spain (Mozo et al 1988). Lie et al (1987) found that plant gene centres of the primitive pea (Pisum sativum) also showed high genetic variation in the specific Rhizobium strains nodulating peas. As 244 out of the total of 255 species of Aspalathus occur in fynbos (Bond and Goldblatt 1984) it is hypothesized that symbiotic specificity may play a role in the distribution of Aspalathus species in this region and in a manner possibly related to soil factors.

This study examined the ability of selected Aspalathus species to form nodules in soils from within and outside their natural distributions. One site, the Cape Point Nature Reserve is within the distribution of both Aspalathus capensis (Walp.) R.Dahlgr. and A.carnosa Berg. (Dahlgren 1966). However these species rarely co-occur, as a result of different habitat preferences. The possible role of symbiotic specificity in the lack of co-occurrence of these species was examined by growing these plants in various soils and in tubes with various soil solution inocula.

The second study site at Hagelkraal Farm, near Agulhas, is 90km from Cape Point and outside the distribution of both A.capensis and A.carnosa (Dahlgren 1966). This is a region of great edaphic variability (Thwaites and Cowling, in press). A third species

A.crassisepala R.Dahlgr. which grows at this site was included in this study, but as it failed to establish in tube culture no information regarding its symbiotic specificity was obtained. The ability of the Aspalathus species to form nodules in an acid and an alkaline soil from this site was examined in tube culture.

The invasive alien legume, Acacia saligna (Labill.) Wendl. has successfully colonized a wide variety of soils throughout the fynbos biome (Milton and Hall 1981). This species was included in both soil and tube experiments to determine whether this invasive success is partly due to an ability to form effective symbiosis with a wide variety of Rhizobium strains i.e to nodulate in a wide variety of soils.

plants

Seeds of *Aspalathus capensis* and *As.carnosa* were collected at Cape Point Nature Reserve and *As.crassisepala* at Hagelkraal Farm. Seeds of *Ac.saligna* had been previously collected at Pella Research Station. Surface sterilization and initiation of germination was achieved by soaking seeds in concentrated sulphuric acid. Treatment times were as follows (in minutes) : *As.carnosa*, 18; *As.capensis*, 20; *As.crassisepala*, 30; *Ac.saligna*, 30. All traces of acid were removed by 10 rinses with sterile deionized water. Treated seeds were placed on YEMA agar and incubated at 10°C for 7 days, during which germination began. They were then transferred to a 10°C/20°C growth chamber for 2 days, before planting.

soils

Soil was collected from 5 sites, corresponding , in 4 cases, to the sites of seed collection. At the Cape Point Nature Reserve, soil was collected at three sites down a slope of acid sandstone (TMG)-derived soil. The soils are referred to as top (CPT), middle (CPM) and bottom (CPB), corresponding to the presence of *As.capensis* only (CPT), both *As.capensis* and *As.carnosa*(CPM) and *As.carnosa* only (CPB). At Hagelkraal Farm, samples of acid colluvial sand (HAS), where *As.crassisepala* is present and shallow alkaline sand overlying limestone (HLS), where none of the above species are present, were collected. Soil samples were stored at 10°C and pH was measured according to Schofield and Taylor (1955).

Experiment 1.

Pregerminated seedlings of As.capensis, As.carnosa and Ac. saligna were planted in 250ml polystyrene cups, containing CPT, CPM and CPB soils. Each pot contained 3 seedlings and there were 10 replicates of each treatment. Pots were placed in the greenhouse, where the day/night temperature was approximately 25 °C /20 °C and watered every 3 days with deionized water. After 12 weeks, plants were removed and soil washed from the roots. Nodules were counted and shoots oven-dried at 80 °C before weighing.

Experiment 2.

As.capensis, As.carnosa, As.crassisepala and Ac.saligna were grown in boiling tubes, containing a supporting roll of filter paper and plugged with non-absorbant cotton wool (Figure 1). These tube assemblies were set up and autoclaved (121°C for 15 minutes), after which aseptic conditions were maintained for all procedures involving tube contents. 15ml of sterile nitrogen-free rooting solution (Deignan 1984) was added, after which a single pregerminated seedling was placed in each tube. Inocula were made up by thoroughly mixing 50g each of CPT, CPM, CPB, HAS and HLS soils with 50ml sterile deionized water. Mixtures were allowed to settle and 1ml of soil solution was added to each tube, two days after seedlings were placed in the tubes. There were 8 replicates of each treatment. Tubes were placed in the greenhouse, with black cardboard cylinders covering the tube

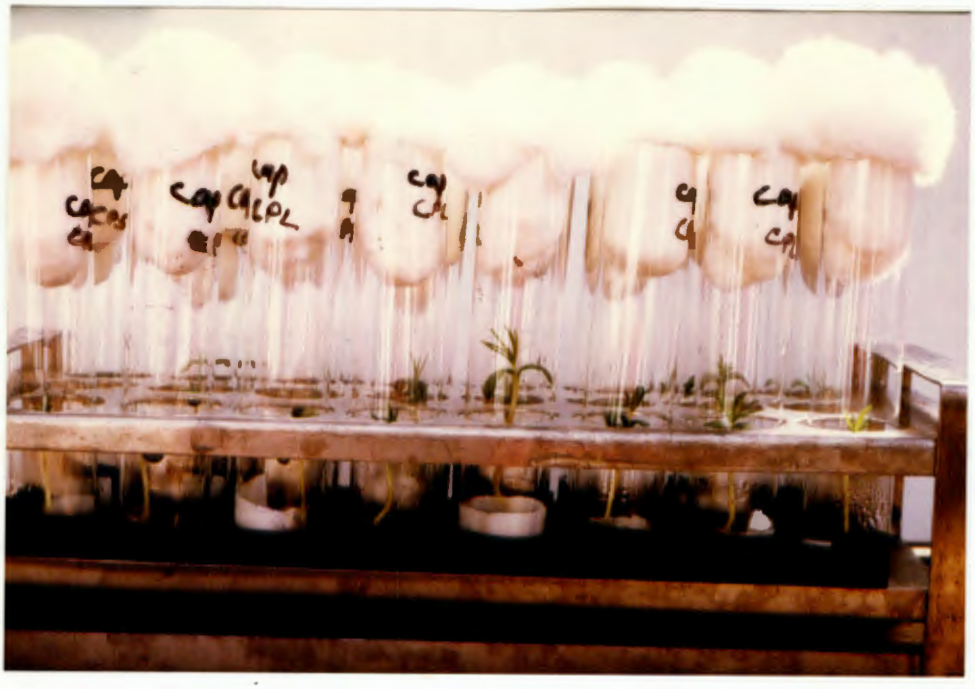

FIGURE 1. Boiling tube assemblies for growing plants in microbiologically-controlled conditions in Experiment 2.

Each tube contains a seedling, supported by a cylinder of filter paper, 15ml nitrogen-free nutrient solution and 1ml soil solution and is sealed by a plug of non-absorbant cotton wool. All tube contents are sterile, except for the seedling which was germinated under aseptic conditions and the soil solution.

lower portions of the tubes. Plants were harvested after 12 weeks. As large differences in nodule size were observed, it was decided to use nodule fresh weight as well as nodule number to measure nodulation success. Nodules were removed and dried on paper towel before weighing. Shoot weight was determined after oven-drying (80°C).

One-way ANOVA and one-way Chi-squared tests, where appropriate were carried out on all results. Where ANOVA showed significant differences ($p<0.05$), multiple range tests were used to define differing means.

soils

The pH of samples was as follows: CPT, CPM and CPB, pH 4.3 ; HAS, pH 4.7 and HLS, pH 7.1.

Experiment 1.

All plant species formed nodules in all soils. Figure 2 shows typical nodules. The largest number of nodules, across all soils was formed by As.capensis (16.05 ± 5.4), followed by As.carnosa (7.04 ± 1.8) and Ac.saligna (4.47 ± 3.0). However there were no significant differences ($p>0.05$) between soils in the mean number of nodules formed or the mean shoot dry weight for any species (Tables 1-3). Ac.saligna in CPM and CPB soils, showed signs of apparent nutrient stress, characterised by yellowing and browning of leaves , relative to the dark green leaves of plants in CPT soil (see figure 3). Plants in CPM soil recovered later in the experiment. Although there were no significant differences in nodule number between soils, A.saligna plants in CPB soil appeared to have smaller nodules, suggesting that the stress symptoms observed were the result of nitrogen deficiency. Thus in the tube experiment nodule weight was determined in addition to nodule number.

Experiment 2.

As.carnosa and As.crasisepala failed to establish in tube culture and so are excluded from the results. As.capensis formed nodules with inocula from CPT, CPM, CPT and HAS soils,

FIGURE 2. Root systems o
soil-grown plants showing
nodules.

a) Aspalathus capensis
b) Apalathus carnosa
c) Acacia saligna

TABLE 1. Number of surviving replicates, mean shoot dry weight and mean number of nodules of soil-grown Aspalathus capensis in three soils.

		Shoot Dry Wt (mg)		No. Nodules	
Soil	n	Mean	S.E.	Mean	S.E.
CPT	5	63.74	1.29	13.80	1.55
CPM	5	69.54	7.78	17.92	2.50
CPB	7	63.10	6.93	16.33	2.44

Key : CPT soil from Cape Point Nature Reserve, top of slope
CPM " " " " " " , midslope
CPB " " " " " " , bottom of slope

TABLE 2. Number of surviving replicates, mean shoot dry weight and mean number of nodules of soil-grown Aspalathus carnosa in three soils. (see soil key in Tab.1.)

		Shoot Dry Wt (mg)		No. Nodules	
Soil	n	Mean	S.E.	Mean	S.E.
CPT	4	38.96	6.52	6.75	1.08
CPM	6	42.04	3.03	6.98	1.06
CPB	8	50.70	1.55	7.24	0.42

TABLE 3. Number of surviving replicates, mean shoot dry weight and mean number of nodules of soil-grown Acacia saligna in three soils. (see soil key in Tab.1.)

		Shoot Dry Wt (mg)		No. Nodules	
Soil	n	Mean	S.E.	Mean	S.E.
CPT	7	39.42	3.43	4.37	0.93
CPM	8	38.76	2.76	4.64	1.10
CPB	7	33.33	3.01	4.37	1.41

FIGURE 3. Acacia saligna in experiment 1. after 10 weeks, showing signs of stress in CPM and CPB soils. (S = CPB soil, L+S = CPM soil, L = CPT soil).

while Ac.saligna nodulated only with inocula from CPT, CPM and CPB soils (Table 4).

The degree of nodulation, measured either by nodule number or nodule fresh weight did not differ significantly ($p>0.05$) between soils for As.capensis (Figure 4, Figure 5 and see appendix for data). However, Ac.saligna showed significantly lower nodule number in CPB soil ($p,<0.05$) (Figure 4). Nodule fresh weight decreased significantly from CPT to CPM soil and from CPM to CPB soil ($p,0.05$) (see appendix for data). Nodulation of A.saligna began earliest in CPM soil, one week before CPT and two weeks before CPB.

Shoot dry weight was not significantly different between soils for either species (Figure 6). As mean values may obscure important effects on individual plants, the effectiveness of nitrogen fixation was estimated by means of a correlation between nodule fresh weight and shoot dry weight for individual nodulated plants. Due to the small number of surviving replicates, it was not possible to obtain an individual correlation for each soil type. As.capensis showed a stronger correlation ($r^2=0.74$; $p<0.005$)) between nodule fresh weight and shoot dry weght across all soils, than did Ac.saligna ($r^2=0.53$; $p<0.01$). The nutrient-stress symptoms observed in Ac.saligna in CPB and CPM soils in the pot experiment did not occur when plants were grown in tubes with CPB and CPM soil solution as inoculum.

TABLE 4. Nodulation of *Aspalathus capensis* and *Acacia saligna* in tube culture with inocula of five soil solutions.

SOIL	*Aspalathus capensis*	*Acacia saligna*
CPT	+	+
CPM	+	+
CPB	+	+
HAS	+	-
HLS	-	-

Key . + nodules formed
- no nodules formed

CPT soil from Cape Point Nature reserve, top of slope
CPM " " " " " " , midslope
CPB " " " " " " , bottom of slope
HAS Hagelkraal acid sand
HLS soil from Hagelkraal limestone outcrop

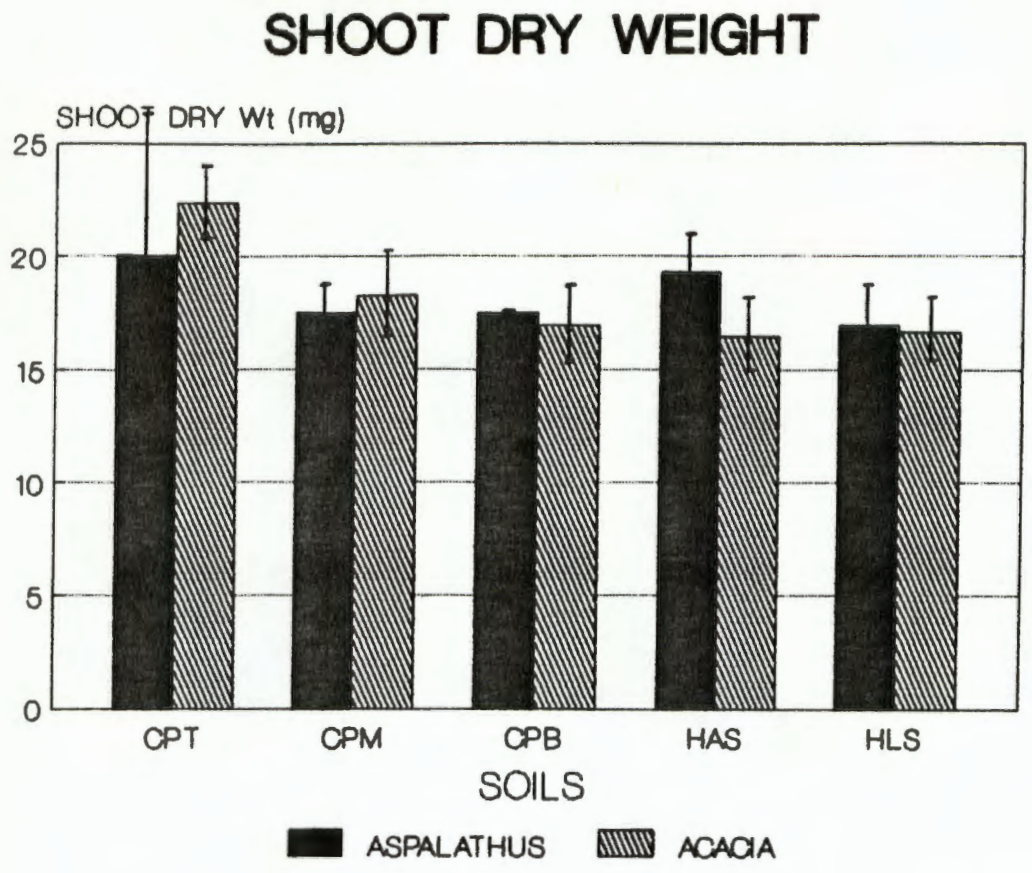

FIGURE 4. Mean shoot dry weight of tube-grown Aspalathus capensis and Acacia saligna, with inocula of five soil solutions. Bars indicate S.E. (for soils see key in Tab.4.)

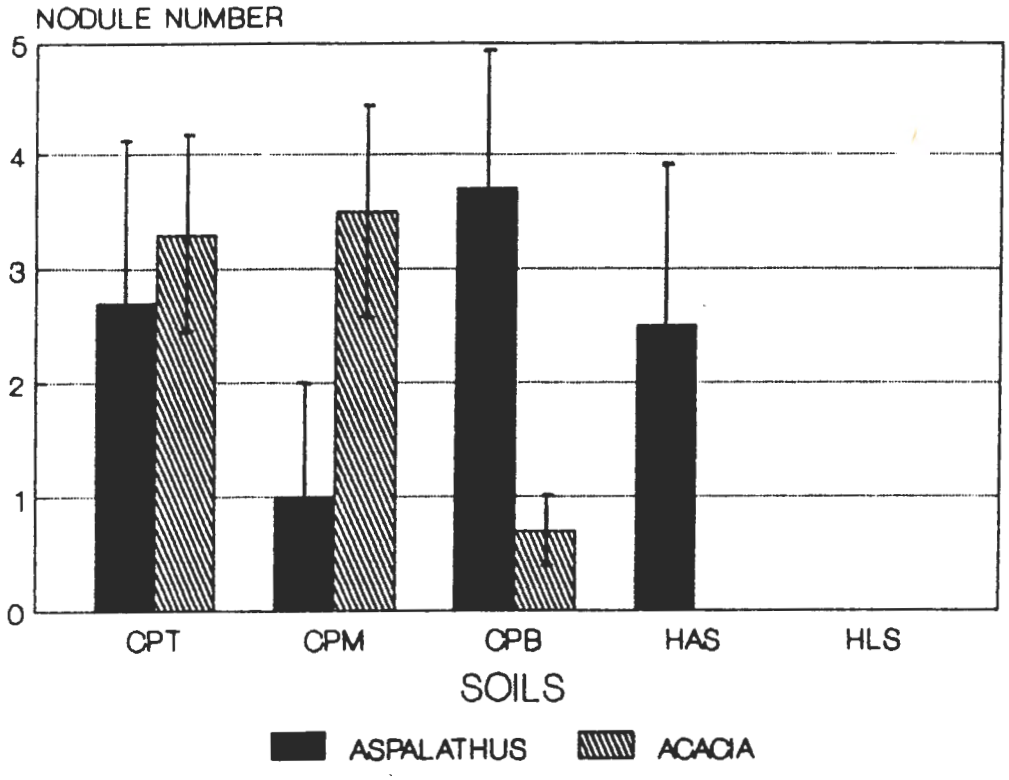

FIGURE 5. Mean nodule number of tube-grown Aspalathus capensis and Acacia saligna , with inocula of five soil solutions. Bars indicate S.E. (for soils see key in tab.4)

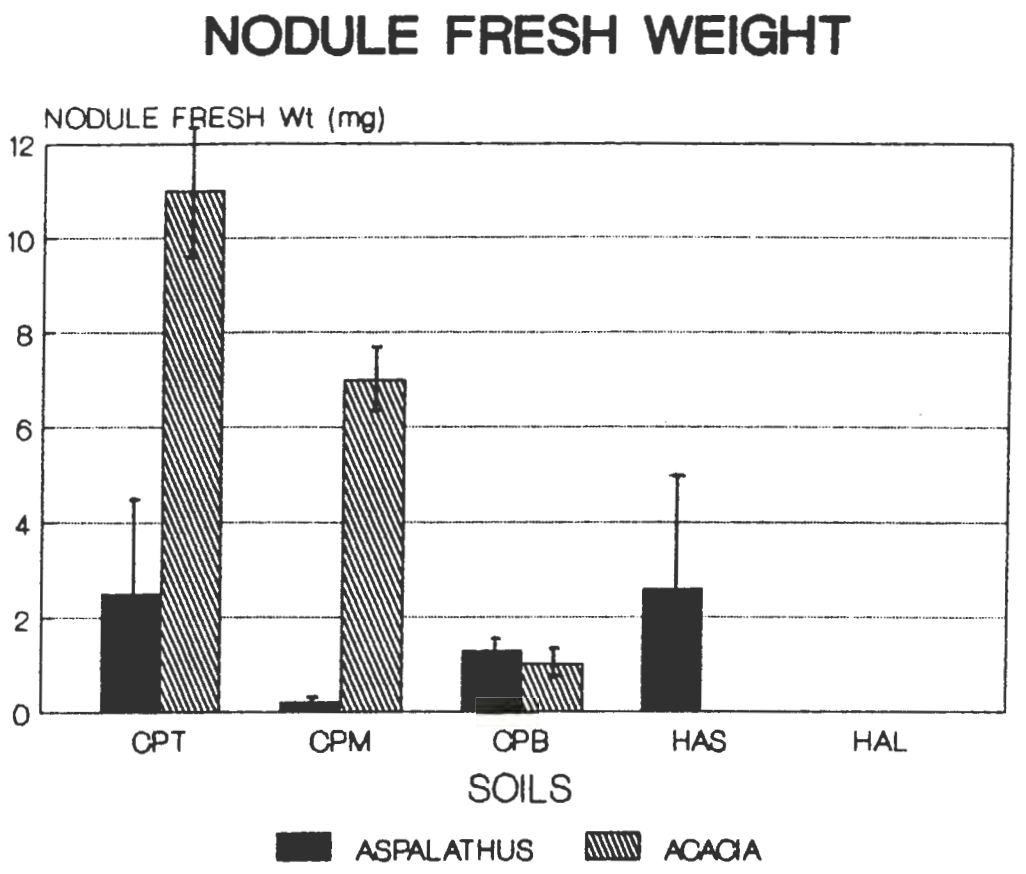

FIGURE 6. Mean nodule fresh weight (per plant) of tube-grown Aspalathus capensis and Acacia saligna, with inocula of five soil solutions. Bars indicate S.E. (for soils see key in tab.4.)

As.capensis is usually found on higher slopes on the Cape Peninsula, close to TMG sandstone outcrops and *As.carnosa* on lower slopes and flats with deeper soil (Dahlgren 1966). The ability of *As.capensis* and *As.carnosa* to form nodules in all 3 soils within their distribution, including sites where either species is absent, suggests that it is not the presence of specific *Rhizobium* strains which result in the different habitat preferences of these species. However, from these experiments, it is not known whether it is the same strain of *Rhizobium* which nodulates both species in all three soils. Studies of natural populations of *Rhizobium* have found great genetic variability in strains from any one site (Glynn *et al* 1985, Broughton *et al* 1987) and selection of particular strains from such populations have been shown for different host species (Robinson 1969) and different host cultivars (Dughri and Bottomley 1984). It is thus possible that *As.capensis* and *As.carnosa* are selecting different species-specific strains from these soils. This could be shown by inoculating both species with *Rhizobium* isolates from nodules formed in each soil.

Restriction of specific strains to soils where the host plant grows naturally, as shown by Lie *et al* (1987) and Mozo *et al* (1988), leads to the suggestion that if *Aspalathus* spp. are highly specific in their symbiosis with *Rhizobium*, they would not nodulate in soils from outside their distribution. Thus the nodulation of *As.capensis* in HAS soil inoculum implies that it is

not highly specific. However, as this experiment made use of soil solutions as inoculum, and not pure Rhizobium cultures, it is not possible to ascertain whether the strains nodulating As.capensis in Cape Point Nature Reserve and at Hagelkraal Farm are, in fact, the same. If it can be shown (for example by means of polyacrilamide gel electrophoresis (PAGE)) that Rhizobium isolates from the two sites are similar, this would mean that As.capensis has symbiotic specificity to a strain or group of strains which occur over an area larger than its current distribution. Such widely distributed strains have been shown by Young et al (1987), who found genetically similar strains of Rhizobium leguminosarum at sites 25km apart and Mozo et al (1988) who showed the presence of highly specific and genetically homogenous strains, nodulating Hedysarum coronarium over a wide geographical region in southern Spain.

On the other hand, if analysis of these strains would show them to be clearly different, this would mean that As.capensis selects a range of heterogenous Rhizobium strains for nodulation. This does not necessarily preclude the possibility that Rhizobium strains may play a role in the distribution of their host plants. Strains of Rhizobium able to nodulate the same host have been shown to differ in their ability to fix nitrogen (May and Bohlool 1983). It is hypothesized that if the strains nodulating A.capensis differ in their effectiveness, the distribution of the host species may be influenced by the distribution of the more effective strains. Thus it would be

expected that nodulating strains from within the host's present distribution are more effective than those from outside . . In this study, plant growth, measured as shoot dry weight was determined in order to give an indication of the effectiveness of the *As.capensis*-*Rhizobium* and *Ac.saligna*-*Rhizobium* symbioses. A significant ($p<0.01$) correlation was shown between nodule fresh weight and shoot dry weight, indicating an effective symbiosis across all soils. However, to obtain estimates of the effectiveness of the *As.capensis*-*Rhizobium* symbioses formed in each soil, it would be necessary to calculate a separate correlation between nodule fresh weight and shoot dry weight for each soil, which was not possible in this experiment because of the low number of surviving replicates. In addition, mean shoot dry weight for *As.capensis* in each soil showed much variability because of non-uniform nodulation within soil types. Similar variability in the symbiotic interactions of wild genotypes of legumes has been shown for *Glycine soja* (Keyser and Kregan 1984). To examine the possibility of heterogeneity in the effectiveness of *Rhizobium* strains nodulating *As.capensis*, larger numbers of replicates and techniques such as acetylene reduction for quantification of nitrogen fixation are required.

The failure of *As.capensis* to nodulate in the limestone-derived HLS soil suggests some degree of specificity in the *Rhizobium*-*As.capensis* symbiosis. Observation of other nodulated legumes on similar limestone outcrops shows that *Rhizobium* strains are present in such soils (pers. obs.). The soils from within the range of *A.capensis* were highly acidic (pH 4.3) as was the HAS

soil (pH 4.7). Thus the HLS soil (pH7.1) may be too alkaline for such acid-adapted strains to survive. However soil pH differences alone may not be sufficient to account for differences in Rhizobium populations. Wood and Shepherd (1987) found little variation in effectiveness and pH tolerance in R.trifolii strains along a transect with pH ranging from 4.5 to 5.6, suggesting some degree of genetic uniformity amongst these strains.

A large proportion of the major invasive alien plants in the fynbos biome are leguminous Acacia spp. (MacDonald and Jarman 1984). Roux and Warren (1966) suggested that symbiotic nitrogen fixation could be a factor favouring the invasion of legume species in fynbos. They showed that Ac.cyclops formed effective nodules with strains of Rhizobium from the Cape Flats. As Ac.saligna is also a major invasive species in fynbos (Macdonald and Jarman 1984) and occurs in a wide variety of soils (Milton and Hall 1981) it was hypothesized to be less specific in its symbiotic requirments than Aspalathus spp., eg. As.capensis. The inability of As.saligna to nodulate in either Hagelkraal soil, therefore requires investigation. Furthermore, differences in various aspects of nodulation of Ac.saligna were observed between the Cape Point soils. Soil-grown plants in CPB and CPM soils showed symptoms of nitrogen stress. Tube-grown plants showed later nodulation and lower nodule number in CPB and lower nodule fresh weight in both CPM and CPB soils. This suggests that differences in the rate of development of the symbiosis and

the rate of nodule growth occur between these soils. As the stress symptoms were not observed in the tube-grown plants, it appears that soil factors other than the microsymbionts are involved. The higher nodule mass in the CPT soil did not, however, result in in significantly higher mean shoot dry weight. This together with the weaker correlation across soils ($p<0.01$, compared to $p<0.005$ for *As. capensis*) between nodule fresh weight and shoot dry weight of nodulated *Ac. saligna*, suggests that the natural strains may be more effective on the indigenous plants than on the introduced plants, as suggested by Devine (1984). It must be noted, however that these experiments deal only with seedlings, up to 12 weeks old. Differences observed in nodulation and growth at this stage may not be important later, as plants may recover from earlier setbacks, as occurred with CPM soil-grown plants.

Evidence of symbiotic specificity of introduced *Acacia* species, was given by Dreyfuss and Dommergues (1981) in Senegal. *A. mearnsii* which is also an invasive species in fynbos, was shown to be nodulated only by slow-growing strains (*Bradyrhizobium*), while other species were nodulated by only fast-growers (*Rhizobium*) and a third group, by both. If other *Acacia* species show similar specificity, the relative proportions of fast- and slow-growing strains could be a factor restricting the invasive ability of these species in certain soils.

Acacia is one of the very small group of genera which include species nodulated by *Rhizobium* strains and by *Bradyrhizobium*

strains (Dreyfuss and Dommergues (1981). The other genera in which this has been reported are, Glycine, Lotus, Lablab and Lupinus (Schlinkert Miller and Pepper 1988). Before the commencement of this study, only Bradyrhizobium strains had been isolated from Aspalathus species (Staphorst and Strijdom 1975, Deschodt and Strydom 1976). However, isolates obtained from the nodules of A.capensis and A.carnosa in experiment 1., were found to be fast-growing, i.e. species of Rhizobium (Diegnan and Law pers. com.). This means that Aspalathus can be added to this list of genera with species nodulated by Rhizobium and by Bradyrhizobium.

This preliminary study showed differences in nodulation of both indigenous and introduced legumes in different soils within and outside their current distributions. However, further work with specific techniques such as PAGE, to distinguish individual strains and cross-inoculation studies using purified Rhizobium isolates, are required to understand in more detail, the role symbiotic specificity may play in the distribution of these legume species.

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REFERENCES

BOND P. and GOLDBLATT P. 1984 Plants of the Cape flora. A decriptive catalogue. J.S.Afr.Bot. Suppl.Vol. 13.

BROUGHTON W.J., HEYCKE N., PRIEFER U., SCHNEIDER G-.M and STANLEY J. 1987 Ecological genetics of Rhizobium meliloti : diversity and competative dominance. FEMS Microbiol.Lett. 40: 245-249.

DAHLGREN R. 1966 Revision of the genus Aspalathus. Part II. The species with ericoid and pinoid leaflets. The Aspalathus carnosa group. Opera Bot. 11: 23-35.

DEIGNAN M.T. 1984 Effects of mineral nitrogen on the Trifolium repens - Rhizobium trifolii symbiosis. Thesis submitted for degree of Ph.D. University of Belfast.

DESCHODT C.C. and STRIJDOM B.W. 1976 Effective nodulation of Aspalathus linearis ssp. linearis by rhizobia from other Aspalathus species. Phytophylactica 8: 103-104.

DEVINE T.E. 1984 Genetics and breeding of nitrogen fixation. In : Biological nitrogen fixation. (M. Alexander, Ed) pp 127-154.

DREYFUSS B.L. and DOMMERGUES Y.R. 1981 Nodulation of Acacia species by fast- and slow-growing tropical strains of Rhizobium. Appl.Env.Microbiol. 41(1): 97-99.

DUGHRI M.H. and BOTTOMLEY P.J. 1984 Soil acidity and the composition of an indigenous population of Rhizobium trifolii in nodules of different cultivars of trifolium subterraneum L. Soil Biol.Biochem. 16(4): 405-411.

EAGLESHAM A.R.J., STOWERS M.D., MAINA M.L., GOLDMAN B.J., SINCLAIR M.J and AYANABA A. 1987 Physiological and biochemical aspects of diversity of Bradyrhizobium sp. (Vigna) from three West African soils. Soil Biol.Biochem. 19(5): 575-581.

GLATZLE A., SCHULTE-BATENBROCH T. and BROCKWELL J. 1986 Symbiotic incompatability between two forage species of Hedysarum , grown in Morocco, and their homologous rhizobia. FEMS Microbiol.Lett. 37: 39-43.

GLYNN P., HIGGINS P., SQUARTINI A. and O'GARA F. 1985 Strain identification in Rhizobium trifolii using DNA restriction analysis, plasmid DNA profiles and intrinsic antibiotic resistances. FEMS Microbiol.Lett. 30: 177-182.

KEYSER H.H. and CREGAN P.B. 1984 Interactions of selected Glycine soja Sieb and Zucc. genotypes with fast and slow-growing soybean rhizobia. Crop Sci. 24: 1059-1062.

LIE T.A., GOKTAN M., ENGIN M., PIJNEBORG J. and ANLARSAL E.

1987 Co-evolution of the legume-Rhizobium association. Plant Soil 100: 171-181.

MACDONALD I.A.W. and JARMAN M.L. 1984 **Invasive alien organisms in the terrestrial ecosystems of the Fynbos Biome, South Africa.** S.Afr.Nat.Sci.Prog.Rep. 85.

MILTON S.J. and HALL A.V. 1981 Reproductive biology of australian acacias in the south-western Cape Province, South Africa. Trans.Roy.Soc.S.Afr. 44(3): 465-485.

MOZO T., CABRERA E. and RUIZ-ARGUESO T. 1988 Diversity of plasmid profiles and conservation of symbiotic nitrogen fixation genes in newly isolated Rhizobium strains nodulating sulla (Hedysarum coronarium). Appl.Env.Microbiol. 54(5): 1262-1267.

ROBINSON A.C. 1969 Host selection for effective Rhizobium trifolii by red clover and subterranean clover in the field. Aust.J.Agric. 20: 1053-1060.

ROUX E.R. and WARREN J.L. 1963 Studies of the autecology of Australian acacias in South Africa. II. Symbiotic nitrogen fixation in Acacia cyclops A Cunn. S.Afr.J.Sci. 59: 294-295.

SCHLINKERT MILLER M. and PEPPER I.L. 1988 Physiological and biochemical characteristics of a fast-growing strain of lupin rhizobia isolated from the Sonoran Desert. Soil Biol.Biochem. 20(3):319-322.

SCHOFIELD R.K. and TAYLOR A.W. 1955 The measurement of soil pH. Soil Sci.Soc.Am.Proc. 19: 164-167.

SPAINK H.P., WIJFFELMAN C.A., PEES E., OKKER R.J.H. and LUGTENBERG B.J.J. 1987 Rhizobium nodulation gene nodD as a determinant of host specificity. Nature 328:337-340.

STAPHORST J.L. and STRYDOM B.W. 1975 Specificity in the Rhizobium symbiosis of Aspalathus linearis(Burm.Fil.) R.Dahlgr. ssp linearis. Phytophylactica 7: 95-96.

TRINICK M.J. 1968 Nodulation of tropical legumes. I. Specificity in the Rhizobium symbiosis of leucaena leucocephala. Exp.Agr. 4:243-253.

WOOD M. and SHEPHERD G. 1987 Characteristics of Rhizobium trifolii isolated from soils of different pH. Soil Biol.Biochem. 19(3): 317-321.

WOOMER P., SINGLETON P.W. and BOHLOOL B.B. 1988 Ecological indicators of natural rhizobia in tropical soils. Appl.Env.Microbiol. 54(5):112-116.

YOUNG J.P.W., DEMETRIOU L. and APTE R.G. 1987 Rhizobium population genetics : enzyme polymorphism in Rhizobium leguminosarum from plants and soil in a pea crop. Appl.Env.

Microbiol. 53(2): 397-402.

YOUSEF A.N., AL-NASSIRI A.S., AL-AZAWI S.K. and ABDUL-HUSSAIN N. 1987 Abundance of peanut Rhizobium as affected by environmental conditions in Iraq. Soil Biol.Biochem. 19(4): 391-396

APPENDIX

TABLE 5. Number of surviving replicates, mean shoot dry weight, mean number of nodules and mean nodule fresh weight of tube-grown Aspalathus capensis with inocula of five soils. (see soil key in Tab.4.)

		Shoot Dry Wt (mg)		No.Nodules		Nodule Fr. Wt (mg)	
Soil	n	Mean	S.E.	Mean	S.E.	Mean	S.E
CPT	3	20.10	4.71	2.67	1.46	2.47	2.08
CPM	3	17.53	1.25	1.00	1.00	0.17	0.17
CPB	3	17.47	0.09	3.67	1.20	1.13	0.28
HAS	4	19.25	1.80	2.50	1.44	2.63	2.37
HLS	3	16.97	1.86	0	0	0	0

TABLE 6. Number of surviving replicates, mean shoot dry weight, mean number of nodules and mean nodule fresh weight of tube-grown Acacia saligna with inocula of five soil solutions. (see soil key in Tab.4.)

		Shoot Dry Wt (mg)		No. Nodules		Nodule Fr. Wt. (mg)	
Soil	n	Mean	S.E.	Mean	S.E	Mean	S.E
CPT	3	22.40	1.66	3.33	0.88	11.13	1.49
CPM	6	18.25	1.86	3.50	0.99	6.9	0.71
CPB	6	16.98	1.74	0.67	0.33	0.6	0.31
HAS	6	16.53	1.53	0	0	0	0
HLS	5	16.60	1.42	0	0	0	0